



**EASTERN REGIONAL RESEARCH CENTER
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
600 E. MERMAID LANE
WYNDMOOR, PA 19038
(215) 233-6400**

Title: Pulsed Electric Field Effects on Bacteria and Yeast Cells

Author(s): D.J. Geveke, and M.F. Kozempel

Citation: Journal of Food Processing Preservation (2003) 27: 65-72

Number: 7293

Please Note:

This article was written and prepared by U.S. Government employees on official time, and is therefore in the public domain.

Our on-line publications are scanned and captured using Adobe Acrobat. During the capture process some errors may occur. Please contact William Damert, wdamert@arserrc.gov if you notice any errors in this publication.

PULSED ELECTRIC FIELD EFFECTS ON BACTERIA AND YEAST CELLS¹

D.J. GEVEKE² and M.F. KOZEMPEL

*Agricultural Research Service
U.S. Department of Agriculture
Eastern Regional Research Center
600 East Mermaid Lane
Wyndmoor, PA 19038*

Accepted for Publication January 29, 2003

ABSTRACT

*Cell suspensions of yeasts and bacteria in the exponential growth phase were subjected to pulsed electric fields of 12.5 kV/cm intensity to study inactivation. Up to 20 pulses of 0.3 ms duration were applied at a maximum temperature of 30°C. The yeasts *Saccharomyces cerevisiae* and *Candida stellata* were reduced by >3 log. These reductions were due to nonthermal effects. Reductions of approximately 1 log or less were obtained for the bacteria *Escherichia coli* and *Listeria innocua*. However, by acidifying the *L. innocua* suspension from pH 6.6 to 3.8, a 3 log reduction was achieved. In contrast, acidification of the *E. coli* suspension had no effect on inactivation.*

INTRODUCTION

The technique of pulsed electric fields (PEF) is receiving a great deal of attention in the quest for alternatives to thermally pasteurizing liquid foods. Microorganisms are inactivated when exposed to high electric field intensities for short durations. Typically, the intensity of the electric fields are on the order of 20 kV/cm and the durations are 1 to 300 μ s. Numerous applications, or pulses, are often required to achieve a significant reduction in microorganisms. Typically, the number of pulses is on the order of 10. Pasteurization occurs at low or moderate temperatures without causing significant sensorial quality changes.

¹ Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

² Correspondence author. TEL: (215) 233-6507; FAX: (215) 233-6406; E-mail: dgeveke@errc.ars.usda.gov

Zimmermann *et al.* (1974) developed a dielectric rupture theory to explain the inactivation of microorganisms in high intensity pulsed electric fields. According to the theory, an external electric field induces a transmembrane electric potential. At sufficient potential, the cell membrane ruptures. Hulsheger *et al.* (1981) treated *Escherichia coli* K12 using high intensity electric field pulses of short duration and reported that the critical intensity was 4.9 kV/cm.

The effectiveness of PEF treatment is dependent on many parameters. Increasing the electric field intensity, duration and number of pulses, and temperature enhances the effectiveness. Microorganisms in the exponential growth phase are more sensitive to PEF than microorganisms in the stationary phase (Barsotti and Cheftel 1999; Jacob *et al.* 1981). In addition, the characteristics of the medium containing the microorganisms, including conductivity and pH, affect the sensitivity (Barsotti and Cheftel 1999; Hulsheger *et al.* 1981).

The type of microorganism affects the inactivation kinetics as well. Generally, yeasts are more sensitive to PEF than Gram negative bacteria which are more sensitive than Gram positive bacteria (Barsotti and Cheftel 1999; Jeyamkondan *et al.* 1999). Hulsheger *et al.* (1983) concluded, however, that yeasts are less sensitive to PEF treatment than Gram negative bacteria when small pulse numbers are applied.

More research is needed on the relative inactivation rates of yeasts and bacteria. The objective of this experimental work was to treat selected yeasts and Gram positive and Gram negative bacteria with PEF using similar growth phase, field intensity, pH, and conductivity conditions to determine the sensitivity of these classes of microorganisms to PEF.

MATERIALS AND METHODS

Strain Maintenance and Culture

Candida stellata (NRRL Y-1446), *Escherichia coli* K-12 and *Listeria innocua* SA3-VT were supplied by C.P. Kurtzman, P.M. Fratamico and L.K. Bagi, respectively, of the U.S. Department of Agriculture. *Saccharomyces cerevisiae* (ATCC 16664) was purchased from American Type Culture Collection (Manassas, VA). The yeasts were grown in yeast malt broth (Difco Laboratories, Sparks, MD) for 48 h at 28°C and the bacteria were grown in brain heart infusion broth (Difco Laboratories) for 24 h at 37°C. Stationary phase cells were dispensed into vials containing 20% glycerol cryoprotectant and frozen for later use.

The frozen precultures were thawed as needed. The yeasts were cultured in yeast malt broth at 28°C. The bacteria were cultured in brain heart infusion at 37°C. Incubation times for the *E. coli*, *L. innocua*, and yeasts were 4 ± 1 ,

5 \pm 1, and 18 \pm 1 h, respectively. These were chosen, based on previous growth curve studies, to reach the exponential growth phase. Growth of the cells was confirmed by optical density measurement at 800 nm.

Sterilized deionized water was inoculated with cultures to give approximately 6 log cfu/mL. In some cases, hydrochloric acid was mixed with the water to reduce the pH, before adding the cultures (Vega-Mercado *et al.* 1996; Wouters 1999). The populations of the untreated cells were not affected by the pH reduction. The physical properties of the cell suspensions are presented in Table 1.

TABLE 1.
PHYSICAL PROPERTIES OF THE CELL SUSPENSIONS

Microorganism	pH	Conductivity, $\mu\text{S/cm}$
<i>C. stellata</i>	5.6	68
<i>E. coli</i>	3.8	96
<i>E. coli</i>	6.3	84
<i>L. innocua</i>	3.8	94
<i>L. innocua</i>	6.6	34
<i>S. cerevisiae</i>	5.2	15

Pulsed Electric Field Treatment

Microorganisms were exposed to pulses of high intensity electric fields, 12.5 kV/cm. A Bio-Rad Gene Pulser (model 1652076; Richmond, CA) was used to generate the pulses. A Bio-Rad Pulse Controller (model 1652098) was used to protect the Gene Pulser at the high field intensities employed. The apparatus is shown in Fig. 1 and consists of a 2.5 kV power supply, a charge and discharge switch, a 3 μF capacitor, a 100 Ω parallel resistor, and a 20 Ω current-limiting series resistor.

Electroporation cuvettes (Invitrogen, Carlsbad, CA) were filled with 250 μL of cell suspension at room temperature. The cuvettes contained flat electrodes separated by a gap of 0.2 cm. One cuvette was used for each treatment to prevent contamination. The electric field was supplied as an exponentially decaying pulse with a peak voltage of 2.5 kV. The field intensity was determined with a Tektronix oscilloscope (model TDS210; Beaverton, OR). The pulse duration was 0.3 ms. A 15 s interval was maintained between pulses to avoid a rise in temperature. Cells were subjected to 2, 5, 10, and 20 pulses at an electric field intensity of 12.5 kV/cm. Controls were not subjected to any electric field, but were in every other way treated the same. Each experiment

was performed in triplicate. Results were expressed as the means of these values \pm the standard deviations.

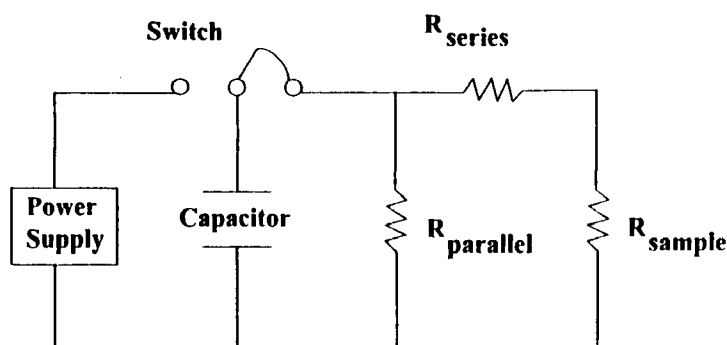


FIG. 1. PULSED ELECTRIC FIELD CIRCUIT

Analysis

Appropriate dilutions of the cell suspensions were plated using a spiral plater (Spiral Biotech model DU2; Bethesda, MD). The bacteria were plated on tryptose agar (Difco Laboratories, Sparks, MD) and incubated for 24 h at 37°C. The yeasts were plated on potato dextrose agar (Difco Laboratories) adjusted to pH 3.5 with tartaric acid (Sigma, St. Louis, MO) and incubated for 48 h at 28°C. Enumerations were made with a colony counter (Spiral Biotech model 500A).

RESULTS AND DISCUSSION

Pulsed electric fields (PEF) inactivate yeasts and bacteria. The extent of microbial inactivation is dependent on the number of pulses (duration of exposure) and the type of microorganism.

Saccharomyces cerevisiae was reduced by 3.3 ± 0.6 log cfu/mL with five pulses of 12.5 kV/cm electric field intensity as presented in Fig. 2. The populations following treatment with higher pulse numbers were below the level of detection, 1 log cfu/mL. Another yeast, *Candida stellata*, was reduced by 3.5 ± 0.2 log cfu/mL with five pulses.

These reductions were achieved at temperatures below 30°C. In addition to experimentally determining the temperature increase, the theoretical maximum temperature increase was calculated. Assuming no heat loss, the temperature rise (in °C) for each pulse is a function of the heat capacity of the fluid, C , and the work density, which is obtained by integrating the power density over time, t :

PEF EFFECTS ON BACTERIA AND YEASTS

$$\Delta T = \frac{0.239}{C} \int_0^\infty \frac{V^2}{Rv} dt \quad (1)$$

where the heat capacity is 1, assuming the fluid is water. The voltage for an exponentially decaying pulse is

$$V = V_{peak} e^{-t/\tau} \quad (2)$$

where V_{peak} is the peak voltage (in V) and τ is the time constant (in s). The resistance is given by

$$R = \frac{d}{A\sigma} \quad (3)$$

where σ is the conductivity (in S/cm) and d is the distance of the gap (in cm). The volume is

$$v = Ad. \quad (4)$$

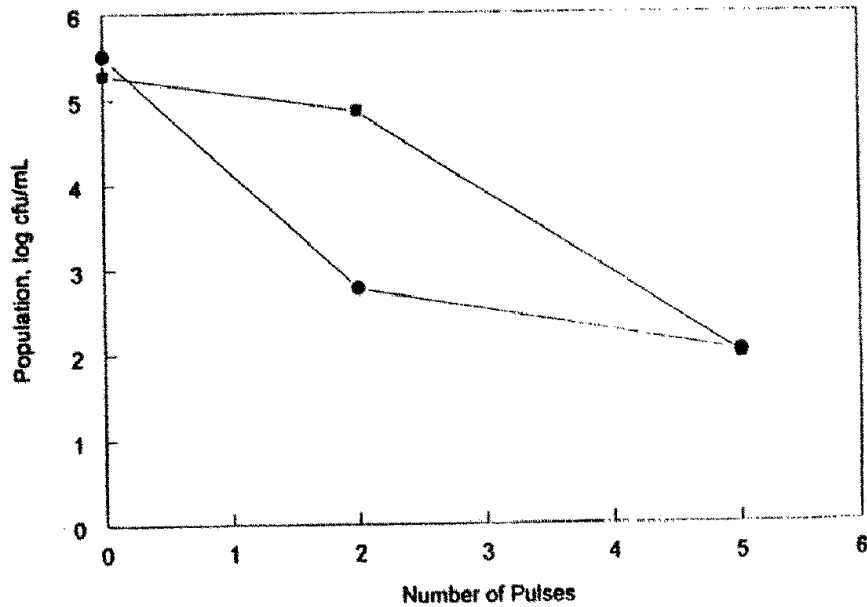


FIG. 2. INACTIVATION OF *SACCHAROMYCES CEREVISIAE* (■) AND *CANDIDA STELLATA* (●) FOLLOWING TREATMENT WITH PULSES OF 0.3 MS DURATION AND 12.5 KV/CM ELECTRIC FIELD INTENSITY
The results are means of data from three experiments.

Substituting Eq. (2) through (4) into Eq. (1) and integrating yields

$$\Delta T = \frac{0.12 V_{peak}^2 \sigma \tau}{d^2} \quad (5)$$

The assessed temperature increases were much less than the calculated values, attributed to the excellent heat transfer provided by the sizeable metal electrodes.

Applying equivalent PEF treatments to bacteria resulted in significantly less inactivation than for the yeasts (critical value of Student's *t* test, $P < 0.01$). This result was expected based on the smaller size of the bacteria (Sale and Hamilton 1968). The population of a suspension of *Escherichia coli* at pH 6.3 was reduced by 1.3 ± 0.4 log cfu/mL after 20 pulses of 12.5 kV/cm electric field intensity (Fig. 3). The treatment was repeated at a pH of 3.8 and the results were not significantly different ($P > 0.05$). Vega-Mercado *et al.* (1996) applied 8 pulses of 40 kV/cm electric field intensity and 2 μ s duration to *E. coli* in simulated milk ultrafiltrate. They determined that inactivation was greater at pH 5.7 than

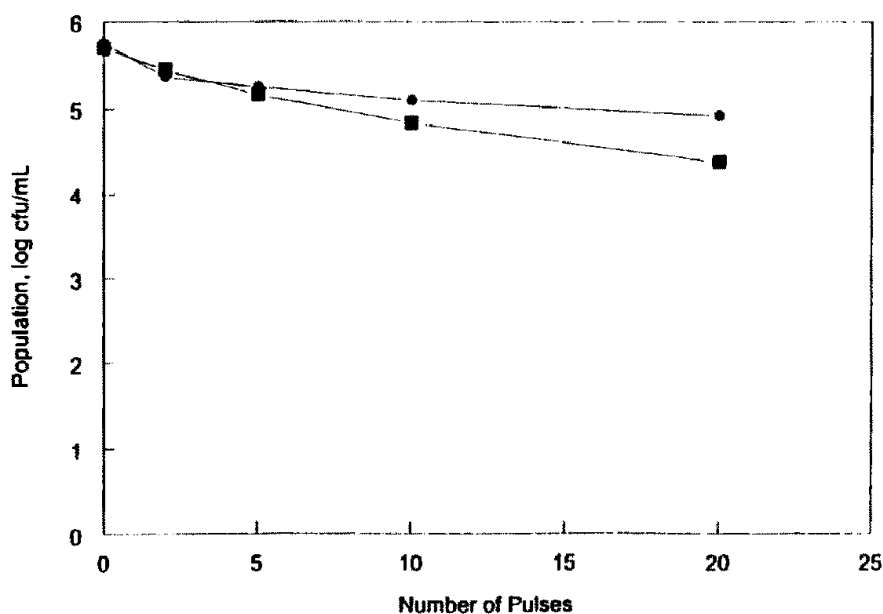


FIG. 3. INACTIVATION OF *ESCHERICHIA COLI* AT pH OF 6.3 (■) AND 3.8 (●) FOLLOWING TREATMENT WITH PULSES OF 0.3 MS AND 12.5 KV/CM
The results are means of data from three experiments.

PEF EFFECTS ON BACTERIA AND YEASTS

at 6.8. The difference in results may be attributable to dissimilarities in electric field intensity, pulse duration, or properties of the suspensions including composition and conductivity. These differing results due to variations in experimental parameters point out the need for standardization of proceedings in an effort to understand the relative susceptibilities of assorted organisms to inactivation by PEF.

Contrary to the results obtained for *E. coli*, the reductions in the populations of *Listeria innocua* were significantly greater at pH 3.8 than 6.6 ($P < 0.05$) (Fig. 4). Wouters *et al.* (1999) reported that PEF inactivation of *L. innocua* above 40°C was greater at pH 4 than at 6. Our results obtained at 30°C extend this finding.

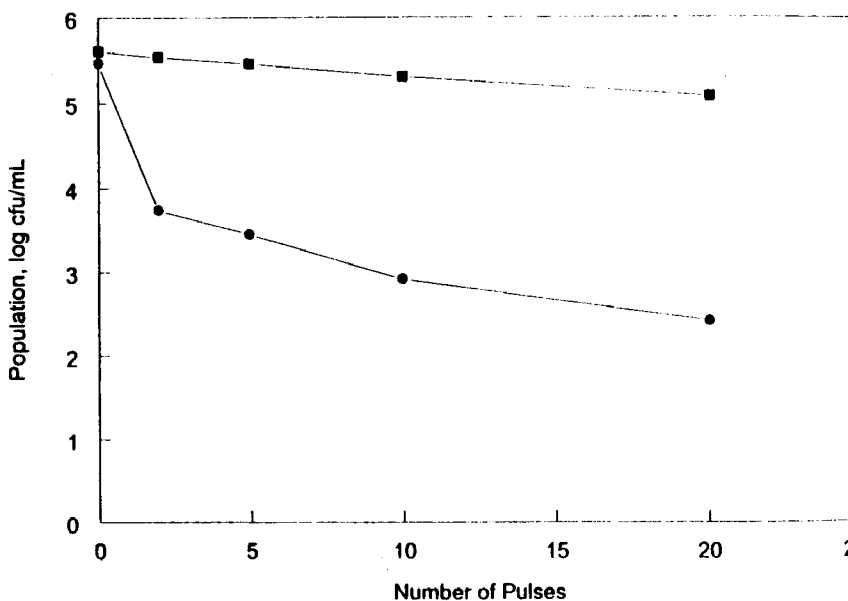


FIG. 4. INACTIVATION OF *LISTERIA INNOCUA* AT pH OF 6.6 (■) AND 3.8 (●) FOLLOWING TREATMENT WITH PULSES OF 0.3 MS AND 12.5 KV/CM
The results are means of data from three experiments.

CONCLUSIONS

Exposure to high intensity pulsed electric fields of 12.5 kV/cm and 0.3 n duration inactivated microorganisms suspended in water. The populations of tl

yeasts *Saccharomyces cerevisiae* and *Candida stellata* were reduced by over 3 log following five pulses of treatment. Inactivation occurred at 30°C and was due to nonthermal effects. Twenty pulses reduced the population of *Escherichia coli* by approximately 1 log. Lowering the pH from 6.3 to 3.8 did not increase the inactivation of *E. coli*, but did increase the inactivation of *Listeria innocua* from greater than 1 log to 3 logs after an exposure of 20 pulses. The results indicate the potential utility of pulsed electric fields to pasteurize liquids at nonthermal conditions.

ACKNOWLEDGMENTS

The authors thank A. Bigley for assistance in performing the experiments, J. Fanelli for microbiological support, C. Brunkhorst (Princeton University) for discussions on electrical engineering aspects, and P.M. Fratamico for lending the electroporation equipment.

REFERENCES

- BARSOOTTI, L. and CHEFTEL, J.C. 1999. Food processing by pulsed electric fields. II. Biological aspects. *Food Reviews Intern.* 15(2), 181-213.
- HULSHEGER, H., POTEI, J. and NIEMANN, E.G. 1981. Killing of bacteria with electric pulses of high field strength. *Radiat. Environ. Biophys.* 20, 53-65.
- HULSHEGER, H., POTEI, J. and NIEMANN, E.G. 1983. Electric field effects on bacteria and yeast cells. *Radiat. Environ. Biophys.* 22(2), 149-162.
- JACOB, H.-E., FORSTER, W. and BERG, H. 1981. Microbiological implications of electric field effects. II. Inactivation of yeast cells and repair of their cell envelope. *Zeitschrift Allgemeine Mikrobiol.* 21(3), 225-233.
- JEYAMKONDAN, S., JAYAS, D.S. and HOLLEY, R.A. 1999. Pulsed electric field processing of foods: A review. *J. Food Prot.* 62(9), 1088-1096.
- SALE, A.J.H. and HAMILTON, W.A. 1968. Effects of high electric fields on micro-organisms. 3. Lysis of erythrocytes and protoplasts. *Biochim. Biophys. Acta* 163(1), 37-43.
- VEGA-MERCADO, H., POTHAKAMURY, U.R., CHANG, F.-J., BARBOSA-CANOVAS, G.V. and SWANSON, B.G. 1996. Inactivation of *Escherichia coli* by combining pH, ionic strength and pulsed electric fields hurdles. *Food Res. Intern.* 29(2), 117-121.
- WOUTERS, P.C., DUTREUX, N., SMELT, J.P.P.M. and LELIEVELD, H.L.M. 1999. Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. *Appl. Environ. Microbiol.* 65(12), 5364-5371.
- ZIMMERMANN, U., PILWAT, G. and RIEMANN, P. 1974. Dielectric breakdown of cell membranes. *Biophys. J.* 14, 881-899.